Mutation profiling of the F508del CFTR allele using haplotype-resolved long-read next generation sequencing

Dario Dilernia[1], Pooneh Amin[1], Julie Flores[1], Arlene Stecenko[1], and Eric Sorscher[1]

[1]Emory University

February 2, 2021

Abstract

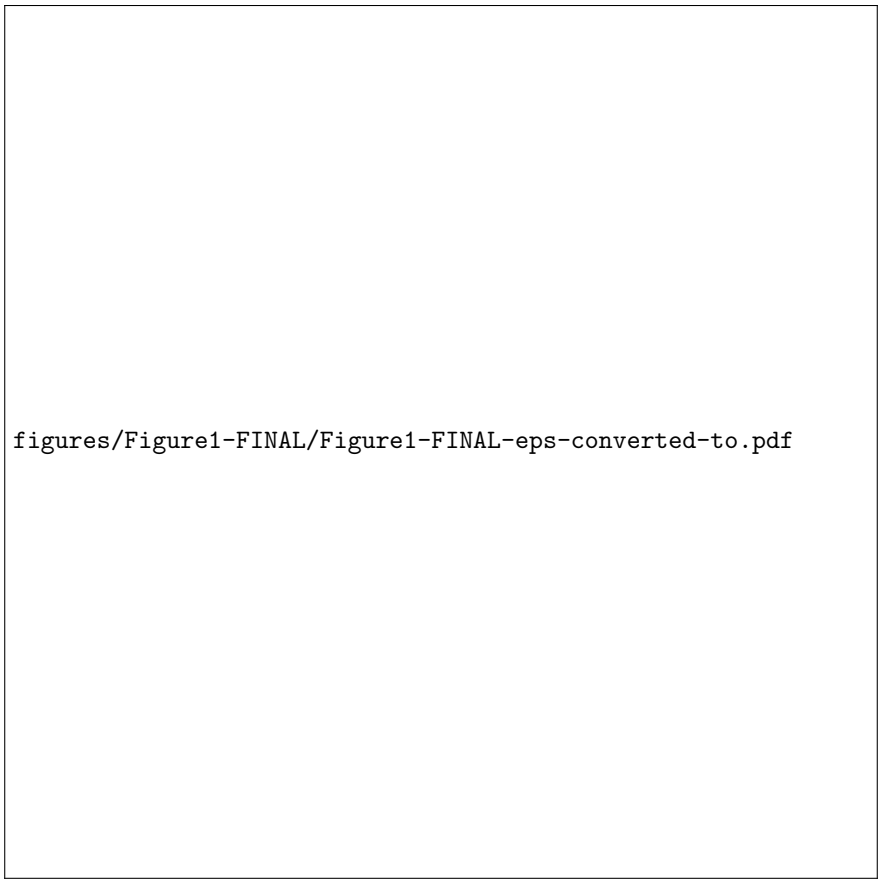
Current approaches to characterize the mutational profile of CFTR are based on targeted mutation analysis (TMA) or whole gene studies derived from short-read next generation sequencing (NGS). However, these methods lack phasing capability which, in certain scenarios, can provide clinically valuable information. In the present work, we performed near-full length CFTR using Single-Molecule Real-Time Sequencing to produce haplotype resolved data from F508del homozygous and F508del compound heterozygous individuals. This approach utilizes target enrichment of the CFTR gene using biotinylated probes, facilitates multiplexing samples in the same sequencing run, and utilizes fully-automated bioinformatics pipelines for error correction and variant calling. We show a remarkable conservation of F508del haplotype, consistent with the single gene founder effect, as well as diverse mutational profiles in non-F508del alleles. By the same method, 105 single nucleotide polymorphisms exhibiting invariant linkage to F508del CFTR (which better define the founder haplotype) were identified. High level homology between F508del sequences derived from heterozygotes, and those obtained from homozygous individuals, demonstrate accuracy of this method to produce haplotype resolved sequencing. The studies provide a new diagnostic technology for detailed analysis of complex CFTR alleles linked to disease severity.

Hosted file

`Manuscript_Dilernia_mainDocument.pdf` available at https://authorea.com/users/393245/articles/506907-mutation-profiling-of-the-f508del-cftr-allele-using-haplotype-resolved-long-read-next-generation-sequencing

Hosted file

`TABLE 1.pdf` available at https://authorea.com/users/393245/articles/506907-mutation-profiling-of-the-f508del-cftr-allele-using-haplotype-resolved-long-read-next-generation-sequencing



Hosted file

Figure2_FINAL.eps available at https://authorea.com/users/393245/articles/506907-mutation-profiling-of-the-f508del-cftr-allele-using-haplotype-resolved-long-read-next-generation-sequencing